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Case Report

Fatal Intoxication with Naftidrofuryl

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Abstract

A 52-year-old man was found dead in his bed. He had financial and psychosocial problems like separation from his wife and children or unemployment due to alcoholism. Under treatment of disulfiram he was presently abstinent from alcohol. As he had suffered from epileptic seizures and dizziness, he received valproic acid and the vasodilator naftidrofuryl, respectively. Autopsy showed no morphologic cause of death. Chemical analysis of blood revealed concentrations for valproic acid and disulfiram in the therapeutic and above the therapeutic range but far below the lethal level, respectively. No ethanol was found. However, the very high concentration of 7500 µg/L naftidrofuryl in whole blood was considered as cause of death, and the most probable manner of death seemed to be suicide. To our knowledge, this is the first reported case of a fatal poisoning with naftidrofuryl.

Keywords: naftidrofuryl, intoxication, death, suicide, fatal

1. Introduction

Naftidrofuryl (Fig. 1) blocks the 5-HT₂ receptors (type 2 serotonin receptors) and thereby inhibits the effects of serotonin-induced vasoconstriction or platelet aggregation. It increases the peripheral circulation of blood, e.g. in peripheral vascular disease (PAOD), Raynaud's phenomenon, cerebrovascular diseases, diffuse circulatory insufficiency in the elderly going along with confusion or behavioral disturbances [1]. Naftidrofuryl is also a stimulator of metabolism by improving aerobic glucose metabolism and by these means preserving cell function in ischemic conditions [2]. Overdosage may result in disturbance of the atrioventricular conduction time, ventricular arrhythmia [3], convulsions, acute renal failure [4] or acute hepatic necrosis [5,6].

In our case we investigated the body of a deceased man. Chemical screening of urine revealed presence of naftidrofuryl; an intoxication with this drug could not be ruled out. Therefore the naftidrofuryl amount was determined in peripheral blood and stomach contents.

2. Case history

2.1 Case history

The decedent was a 52-year-old unemployed man who lived in separation from his wife. He was a chronic alcoholic under treatment with disulfiram and had been abstinent for the last months. Moreover he had suffered from epileptic seizures but was free from convulsions receiving valproic acid. As he complained about dizziness, he was additionally treated with naftidrofuryl. One day he was found dead in his bed by his wife. Coroner's inquest revealed no injuries except some scratches on the right hand, probably caused by the cat living in the same apartment. No loss of urine or feces was noticed. The medical examiner estimated that death had occurred about 12 hours before.

2.2 Autopsy findings

External examination of the body (181 cm/ 74,5 kg) in the Institute of Legal Medicine in Zurich revealed a tongue wound on its right side. The hands were clenched to fists. In the stomach we found 600 ml aqueous content with somewhat digested capsules. We determined a total amount of 2.1 grams of naftidrofuryl (calculated as free base, corresponding to 13 capsules) in the stomach. The heart was slightly too heavy (440 g; according to [7] the normal weight in relation to body length is 300 –380 g) and showed a middle to high degree coronary sclerosis. Histological investigation of a plaque in the right coronary artery revealed a small accumulation of erythrocytes in the plaque, the lumen of the artery however still being widely open. Histology of myocard did not show any pathological alterations. The vessels of the body were sclerotic. The lung (2050 g) was edematous and the liver (1950 g) showed lymphocytic infiltrations which were strictly limited to the portal areas.

3. Materials and methods

3.1 Routine toxicology testing

Routine toxicology testing consisted of blood alcohol analysis by gas chromatography-flame ionization detection followed by Immunoassays RIA DPC (for free morphine and cocaine metabolite in blood) and EMIT d.a.u. (ETS) (for opiates, cocaine metabolite, amphetamine/methamphetamine, methadone, barbiturates, and benzodiazepines in urine). To urine (10 mL), buffer (acetic acid/sodium acetate, pH 4.5, 10 mL) and beta-glucuronidase/arylsulfatase (100 μ L, Roche Diagnostics) were added. The mixture was shaken for 48 hours at 37 °C to achieve enzymatic hydrolysis of conjugated compounds. The mixture was set to pH 2 with tartaric acid, then extracted five times with t-butyl-methyl-ether (totally 170 mL). The combined extracts containing the acidic-neutral fraction of urine were evaporated to dryness, then reconstituted with methanol (500 μ L) for HPTLC. The aqueous fraction was adjusted to pH 8.8–9.0 by adding ammonia (10 %)/ammonium chloride (saturated solution), then extracted 4 times with dichloromethane/methanol (20:1 v/v, totally 140 mL). The combined extracts containing the basic-amphoteric fraction of urine were evaporated to dryness and then reconstituted with methanol (100 μ L) for HPTLC and GC-MS.

Two dimensional HPTLC screenings of the acidic-neutral and the basic-amphoteric fraction were done according to [8] and [9], respectively. UV/VIS remission of spots were taken with a CAMAG TLC scanner 3.

Further qualitative screening of a part of the basic-amphoteric fraction corresponding to 0.1 mL of urine was done on a Varian Saturn 4D GC-MS System (full-scan EI mode, scan range m/z 41–500, helium as carrier gas, flow rate 1.5 mL/min, column J&W DB-5ms, 30 m x 0.25 mm i.d. with 0.25 μ m film, programmed from 120 °C to 280 °C at 20 °C/min, initial hold time 2 min).

3.2 Quantitation of naftidrofuryl in blood

An Isolute HXC 300 mg solid-phase extraction (SPE) column was conditioned with methanol (2 mL) and phosphate buffer (pH=6, 2 mL) under vacuum at a flow rate of 2 mL/min. Peripheric blood (2 mL) was diluted with phosphate buffer (pH=6, 12 mL) and applied to the column. The flow rate was reduced to 1.5 mL/min. After washing with water (1 mL) and 0.01 M acetic acid (0.5 mL), the column was dried for 4 min under vacuum. 50 μ L of methanol were applied to the column and again the column was dried as before for 1 min. The column was centrifuged to completely remove solvents. Further washing was done with acetone/chloroform (1:1 v/v, 4 mL). The fraction eluted with dichloro-methane/isopropanol/ammonium hydroxide (8:2:0.5 v/v, 2 mL) was collected, evaporated to dryness and then reconstituted with 50 μ L of methanol. Quantitation was done with 1 μ L of the reconstituted extract on a Carlo Erba GC equipped with a CTC A200S Autosampler, a DB-1 column (15 m x 0.53 mm i.d. with 1.5 μ m film), and a flameless nitrogen-phosphorus detector (NPD, splitless injection, injector: 280 $^{\circ}$ C; column: 2 min isothermic at 170 $^{\circ}$ C, then programmed from 170 $^{\circ}$ C to 220 $^{\circ}$ C at 5 $^{\circ}$ C/min and from 220 $^{\circ}$ C to 270 $^{\circ}$ C at 25 $^{\circ}$ C/min, final hold time 4 min; detector: 300 $^{\circ}$ C). Retention time for naftidrofuryl was 13.1 min. Methadone (retention time 4.9 min) was used as an internal standard for general performance control at a concentration of 100 ng/mL blood. Calibration was done with methanolic solutions of naftidrofuryl oxalate (Lipha) at concentrations corresponding to 12.5 ng/ μ L and 314 ng/ μ L free naftidrofuryl base. A forced zero two point calibration curve ($R^2=1$) was obtained from three injections (1 μ L) of each calibrator level. The upper calibrator level was chosen to give a peak area approximately corresponding to that of the extract of the autopsy blood. Recovery was 90 percent as determined from a spiked negative testing blood sample (2 mL) with 314 ng/mL naftidrofuryl (calculated as free base), extracted in the same manner as described above for autopsy blood. The method LOD [10] was 30 μ g/L blood and LLOQ was 75 μ g/L blood. ULOL was not determined as the naftidrofuryl concentration in the autopsy peripheric blood was within the linear range. Accuracy (bias) was within \pm 15 % of nominal value, precision within 15 % R.S.D.

Only a limited volume of peripheral blood was available from autopsy. Therefore, we used heart blood for the quantitation of disulfiram, mefenamic acid and valproic acid.

3.3 Quantitation of disulfiram in blood

Disulfiram and its metabolites were cleaved to carbon disulfide by addition of phosphoric acid (20 %, 1 mL) to a headspace vial containing heart blood (1 mL) and distilled water (1 mL) and heated (80 °C, 60 min). For calibration, samples of heart blood tested negative for disulfiram were spiked with known amounts of disulfiram (1000 µg/L, 500 µg/L and 100 µg/L) and treated by the same way. Carbon disulfide was measured by headspace GC-MS (Varian Saturn 4D, fullscan mode, scan range m/z 40–200, helium as carrier gas, flow rate 1.8 mL/min, column GS-Q (J&W Scientific), 30 m, 0.32 µm i.d., starting temperature 60 °C, held isothermic for 2 min., heating rate 20 °C/min, final temperature 200 °C, held for 10 min) comparing the peak areas of mass m/z 76 of the case samples and the calibration samples. The result was calculated as disulfiram equivalents. As 1 mole of disulfiram produces 2 moles of carbon disulfide, 1 µg of disulfiram (molecular mass 296.5) corresponds to 0.51 µg of carbon disulfide (molecular mass 76.1). An analogous procedure, but with water (1 mL) instead of phosphoric acid was used to determine the content of free carbon disulfide in heart blood (calculated as disulfiram equivalents). Negative controls for total carbon disulfide were done with blood samples tested negative for disulfiram, distilled water and phosphoric acid as described above, for the negative controls of free carbon disulfide no phosphoric acid was added.

3.4 Quantitation of mefenamic acid in blood

Heart blood (2 mL) was treated by ultrasound for 10 min. Proteins were precipitated by addition of hydrochloric acid (0.1 N, 12 mL) and vortex mixing followed by centrifugation at 3000 rpm for 10 min. The supernatant was transferred to an Isolute HCX SPE column (300 mg/10 ml), pretreated with methanol (2.5 mL) and hydrochloric acid (0.1 N, 2.5 mL). The column was washed with hydrochloric acid (0.1 N, 4 mL) and hexane (3 ml). The column was then dried under vacuum for 5 min. Mefenamic acid was

eluted with t-butyl-methyl-ether. The extract was evaporated to dryness under nitrogen. The sample was reconstituted in 50 μ L of methanol and analyzed by HPLC on a Dionex LC system with diode array detection (DAD) on a Waters Spherisorb OD/CN column (5 μ m, 150 x 4.6 mm) with 10 μ L of the sample at a detection wavelength of 230 nm. Retention time for mefenamic acid was 10.7 min. For calibration, standards of mefenamic acid (41.0 ng/ μ L and 66.7 ng/ μ L) were used.

3.5 Quantitation of valproic acid in blood

Three samples of heart blood (1 mL each) were analyzed as follows: chloroform (0.5 mL) followed by hydrochloric acid (3 M, 1 mL) was added under vortex mixing. After centrifugation (3000 rpm, 15 min), the chloroform phase was separated from the mixture.

Three samples of blood tested negative for valproic acid (1 mL each) were spiked with 99 μ g of valproic acid and were used as calibrators and treated as described above for the heart blood samples. As negative control, an unspiked blood sample (1 mL) was treated the same way.

All chloroform extracts were analyzed by GC-MS (Varian Saturn 4D, fullscan mode, scan range m/z 41–249, helium as carrier gas, flow rate 1.5 mL/min, column DB-5ms, 30 m, 0.25 μ m i.d., starting temperature 100 °C, held isothermic for 2 min., heating rate 20 °C/min, final temperature 280 °C, held for 20 min). Valproic acid had a retention time of 5.00 min and was identified by its mass spectrum. For quantitation, the peak area of mass m/z 73 (base peak) was determined. Valproic acid content was then calculated from the mean peak area value of the three heart blood samples where the mean peak area value of the three calibrator samples corresponded to 99 μ g. No signal for valproic acid was detected in the negative control.

3.6 Quantitation of naftidrofuryl and mefenamic acid in stomach contents

At the autopsy, a total of 600 mL stomach contents was collected. A pH of 4.0 was observed. The stomach contents consisted of a supernatant yellowish liquid over a layer of beige mud, covering a layer of small microgranules with pink colour.

An Isolute HCX 300 mg solid phase extraction column was conditioned as described above for the quantitation of naftidrofuryl. 3 mL of the homogenized stomach contents were diluted with phosphate buffer (pH=6, 18 mL) and applied to the column. The flow rate was reduced to 1.5 mL/min. After washing with water (1 mL) and 0.01 M acetic acid (0.5 mL), the column was dried for 4 min under vacuum. 50 μ L of methanol were applied to the column and again the column was dried as before for 1 min. The column was centrifuged to completely remove solvents. With acetone/chloroform (1:1 v/v, 4 mL), the fraction of neutral and acidic compounds (Fraction 1) was eluted, collected, and taken to dryness. Finally, the fraction of basic and amphoteric compounds (Fraction 2) was eluted from the column with dichloro-methane/isopropanol/ammonium hydroxide (8:2:0.5 v/v, 2 mL) and taken to dryness.

Fraction 1 was dissolved in 150 μ L of methanol (solution 1). For quantitation of mefenamic acid, 20 μ L of solution 1 were diluted with 100 μ L of methanol. Samples of 1 and 2 μ L of the dilution were applied to TLC plates silica gel F₂₅₄ (20 x 10 cm, Merck, Darmstadt/Germany), alternating with calibrators of 100, 200, 400, 600, and 800 ng mefenamic acid. The plate was developed in a mixture of toluene, t-butyl-methyl-ether, glacial acetic acid, and methanol (60:30:9:1 v/v/v/v). Identification and quantitation of mefenamic acid were done with a CAMAG TLC scanner 3. Mefenamic acid was detected at an R_f-value of 0.74 by its UV remission spectrum. Quantitation was done at a wavelength of 350 nm (polynomial regression, $r = 0.99757$ via height and $r = 0.99778$ via area).

By qualitative HPTLC with identification of the compounds by their UV remission spectra it was found that naftidrofuryl was detectable as well in fraction 1 as in fraction 2.

The total naftidrofuryl content of the stomach contents had therefore to be determined as sum of the quantitations in the two fractions. These quantitations were done in a similar way to that described for mefenamic acid, but with development of the TLC plates in a mixture of methanol and ammonia (99:1 v/v). Naftidrofuryl was identified by its UV remission spectrum at an R_f-value of 0.52–0.58. Quantitation was done at a wavelength of 283 nm.

Fraction 1: 10 µL of solution 1 (see above) were diluted with 1190 µL of methanol. 0.5 and 1 µL of the dilution were applied to a TLC plate as described above, alternating with naftidrofuryl calibrators of 100, 200, 400, 600, and 800 ng. Polynomial regression curve: $r = 0.99318$ via height, $r = 0.99866$ via area. Fraction 2: 10 µL of a solution of fraction 2 in 150 µL of methanol were diluted with 590 µL of methanol. 0.5 and 1 µL of the dilution were applied to a TLC plate, calibrators were as described for fraction 1. Polynomial regression curve: $r = 0.99934$ via height, $r = 0.99942$ via area.

4. Results from drug analysis

No alcohol was detected in the blood and all immunoassays for routine toxicology testing were negative. The chemical screening of the acidic-neutral and of the basic-amphoteric urine fraction revealed the presence of naftidrofuryl, its desethyl metabolite, mefenamic acid and two of its metabolites, and of valproic acid. We also found caffeine, theobromine, nicotine, cotinine and hydroxycotinine in urine which were considered to be of no toxicological relevance. The results of quantitative determination of naftidrofuryl, disulfiram, valproic acid and mefenamic acid are listed in Table 1. Mass spectrum and GC-NPD chromatogram of naftidrofuryl are shown in Figure 2 and 3 (a-d), respectively. Naftidrofuryl concentration in peripheral blood was found to be 7500 µg/L, which is 15 times higher than the higher level of the therapeutic range of 500 µg/L plasma [5]. Disulfiram was measured in heart blood as disulfiram equivalents. This concentration (900 µg/L) was above the therapeutic range of 50 to 400 µg/L serum, but far below the lethal level of 8 mg/L. The concentration of valproic acid and of mefenamic acid in heart blood was 50 mg/L and 6.8 mg/L, respectively, in accordance with therapeutic levels. In stomach contents we found 2.36 mg naftidrofuryl per mL in the fraction of neutral and acidic compounds (fraction 1) and 1.16 mg per mL in the fraction of basic and amphoteric compounds (fraction 2).

5. Discussion

The autopsy of the body of the 52-year-old man did not reveal a morphologically visible cause of death. The heart was slightly too heavy which does not explain death. We observed a coronary sclerosis and in a plaque of the right coronary vessel we found a fresh assembly of erythrocytes. However the lumen was still wide and there was no relevant obstruction for blood flow. Histological examination of myocard was namely inconspicuous and did not show any fresh ischemic lesions. We therefore excluded a coronary heart death. The liver showed a chronic inflammation of the portal areas which we attributed to chronic alcohol consumption.

Analysis of blood showed the presence of naftidrofuryl, disulfiram, valproic acid and mefenamic acid. Disulfiram inhibits selectively the action of acetaldehyde dehydrogenase in the liver [1]. When taken together with alcohol, the concentration of acetaldehyde in blood increases, which can lead to flushes, headache, nausea, dizziness, tachycardia, hypotension or even collapse. The symptoms develop 5 to 10 minutes after intake of alcohol and can last several hours. This unpleasant reaction prevents patients under disulfiram treatment from consuming alcohol. Disulfiram is delivered as dispersible tablets containing 400 mg disulfiram. Normal dosage for maintenance therapy is half a tablet every day or one tablet every second day. 90% percent of an oral dose of the highly lipid soluble disulfiram is absorbed and completely reduced within 4 minutes to diethyldithiocarbamate, which is thought to be the pharmacologically active principle. It is further metabolized to methyldiethyldithiocarbamate, carbon disulfide and diethylamine. Their concentrations and their elimination half-life vary highly from subject to subject [11].

Valproic acid is a well-known antiepileptic agent, used especially to treat generalized seizures [1]. The dosage depends on weight and age. The therapeutic concentration in plasma is 40-150 mg/L [12]. Massive overdosage results in coma and can lead to death, however prognosis of overdosage is good.

Mefenamic acid is a non-steroidal analgetic. Not more than 2 g per day of mefenamic acid should be taken. A serum level of 2 to 20 mg/L is considered to be therapeutic [12]. Overdosage leads to seizures and myoclonus.

Our toxicological investigation revealed a very high concentration of naftidrofuryl in blood as the only relevant finding. The blood levels of the other substances i.e. disulfiram, valproic acid and mefenamic acid were far below lethal concentrations and none of these substances is known to show any interaction with naftidrofuryl.

Naftidrofuryl is available as capsules in Switzerland; together with the facts that such a capsule contains 200 mg naftidrofuryl oxalate (corresponds to 162 mg free base), that three capsules a day are a usual therapeutic dose [1], that a single oral administration of 200 mg naftidrofuryl oxalate results in a peak plasma concentration of approximately 275 µg/L [13], and that plasma levels up to 500 µg/L are considered as therapeutic [12], the very high concentration of 7500 µg/L naftidrofuryl in blood in our case is to consider as toxic. Taking further into account the not yet resorbed toxic potential of 2.1 g naftidrofuryl found in the stomach contents, we conclude that a naftidrofuryl overdose must be the cause of death. To the best of our knowledge this is the first report of a fatal naftidrofuryl poisoning.

Naftidrofuryl is known to cause cardiac conduction problems and convulsions when taken in higher amounts. As sign of convulsions we found a bite in the tongue. It seems that the decedent suffered from terminal seizures although he was under treatment with valproic acid and although its blood level was in the therapeutic range. Apparently, the valproic acid level was not high enough to prevent naftidrofuryl-induced seizures.

The police closed the case assuming suicide since it is unlikely that the high concentration of naftidrofuryl in the blood and in the stomach contents results from an accidental intake of such a huge amount of naftidrofuryl. The motive for the suicide may have layed in a difficult psychosocial situation of the man.

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Table

Table 1: Drug concentrations in blood and stomach contents

	Concentrations		
	Peripheral blood (mg/L)	Heart blood (mg/L)	Stomach Contents (mg/L)
Ethanol	0.0	n.m.	n.m.
Naftidrofuryl	7.5	n.m.	3500
Valproic acid	n.m.	50	n.m.
Mefenamic acid	n.m.	6.8	100
Total carbon disulfide ^a	n.m.	0.9	n.m.
Free carbon disulfide ^a	n.m.	0.14	n.m.

n.m. – not measured

^a calculated as disulfiram equivalents

Figures

Fig. 1. Structure of naftidrofuryl.

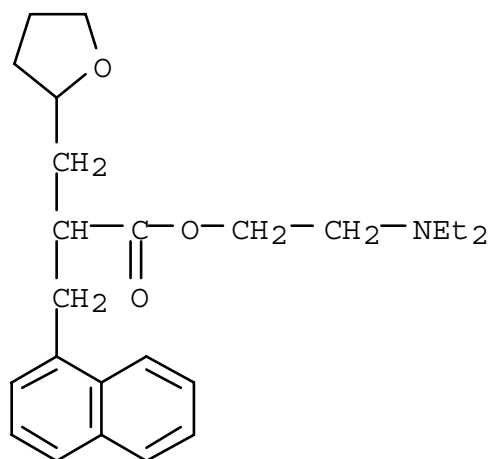


Fig. 2. Mass spectrum of naftidrofuryl ($M = 383$; m/z 384 ($M+1$)⁺).

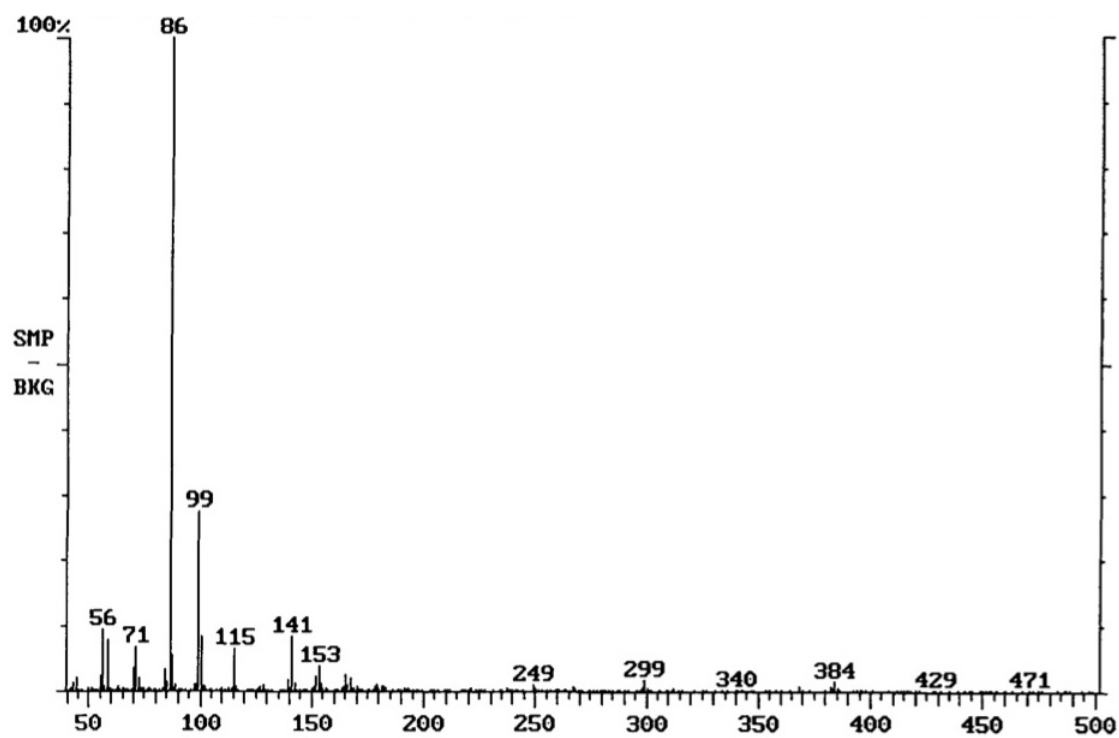
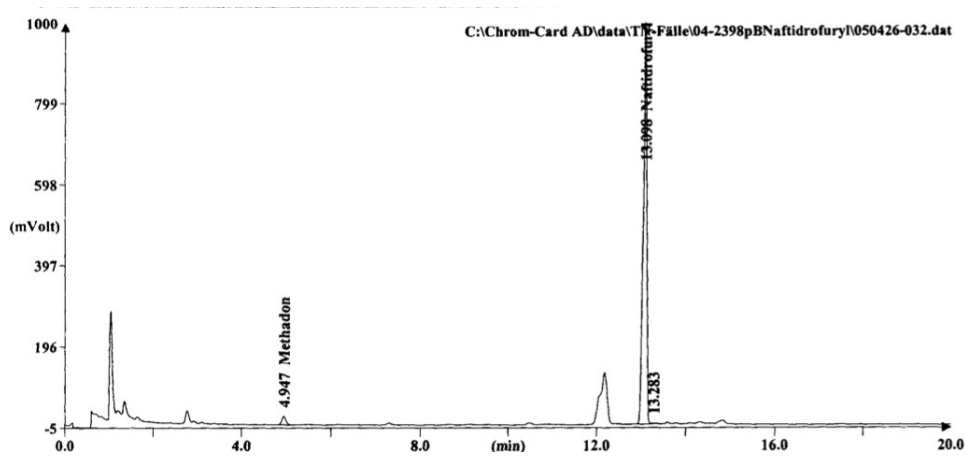
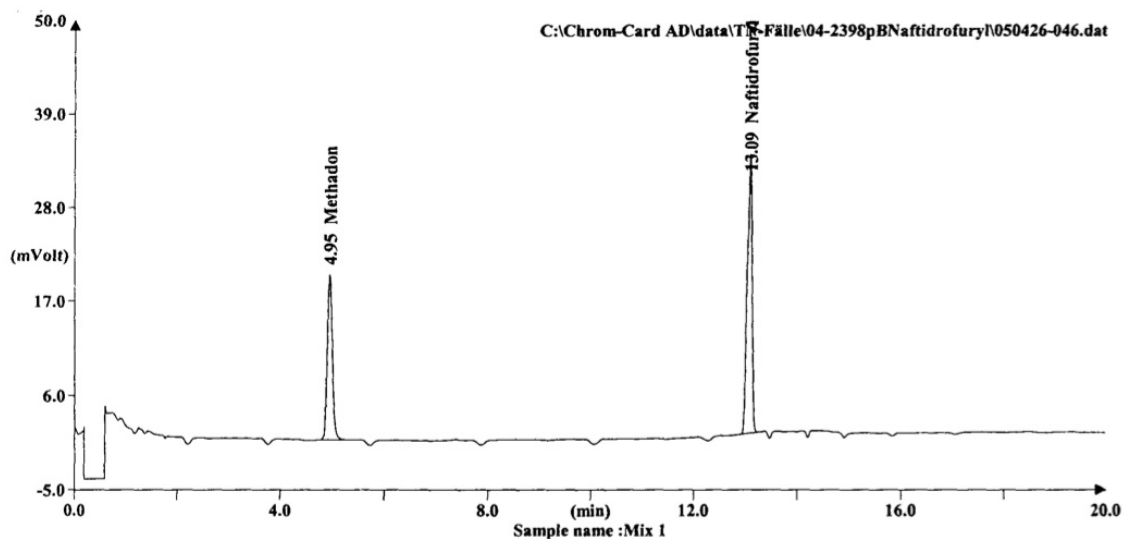


Fig. 3. Chromatograms (GC-NPD).

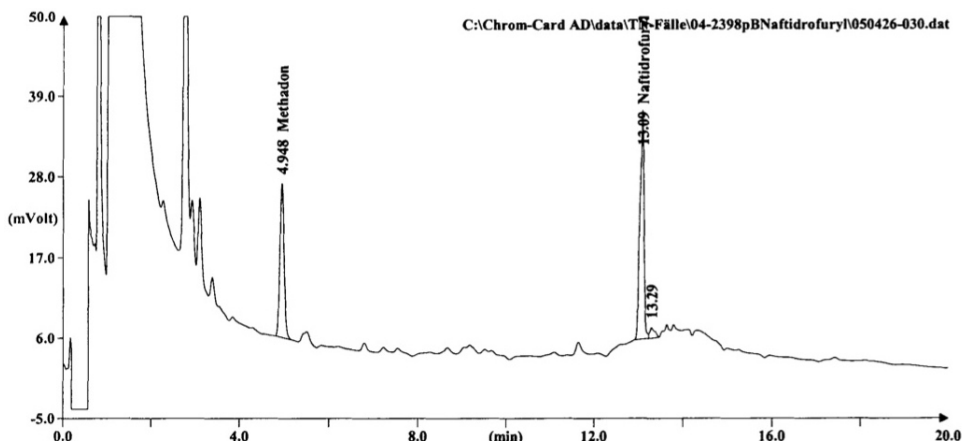
a. Extract of peripheric blood from autopsy (2 mL), injected 1/50 (1 μ L). Naftidrofuryl: t_R = 13.098 min, methadone (IS): t_R = 4.947 min.



b. Calibrator mixture (3.8 ng methadone and 12.5 ng naftidrofuryl).



c. Extract (injected 1/50 (1 μ L methanolic solution)) of a blood sample (2 mL), tested negative for methadone and naftidrofuryl, spiked with 95 ng methadone and 314 ng naftidrofuryl.



d. Extract (injected 1/50 (1 μ L)) of a blood sample (2 mL), tested negative for methadone and naftidrofuryl, spiked with 95 ng methadone only (negative control for naftidrofuryl).

